

iGEM TU/e 2014

Biomedical Engineering

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FACS - Antibody Titration

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1 Labelling the Anti-HA antibodies with 5/6-TAMRA

- Determine the concentration of the Hemagglutinin (HA) Monoclonal Antibody, Mouse (5B1D10) with UV-VIS (Nanodrop)
- Add \pm 30:1 of the stock solution 5/6-TAMRA-NHS ester to the stock of antibody
- Wrap in aluminium foil and put in the fridge overnight
- Wash two times with PBS in 10 kDa centrifuge tubes for 10 min, 4000 rpm, 4°C, pipet up and down before adding new PBS
- Pipet up the residual from the filter
- Determine the concentration and ratio antibody:5/6-TAMRA using UV-VIS (Nanodrop)

2 Stock solutions

- 4.52 μ M HA Anti Mouse in PBS
- 1 μ M antibody HA Anti Mouse in PBS
- 200 nM antibody HA Anti Mouse in PBS
- 5 mM DBCO-PEG4-5/6-TAMRA
- Buffers: PBS (DBCO) or PBS-0.1%BSA (antibody)

3 Preparation of FACS samples

- Prepare following tubes:

Tube	[DBCO]	Cells (10 ⁹)	DBCO volume to add (μL)		DBCO/tag ratio
			5 mM		
1	0	200 μL			
2	30 μM	200 μL	1.21		182.2

- React DBCO tubes for 1h in shaking block at 4°C
- Prepare FACS samples:
 - Spin down the cells for 10 min at 13,400 rpm
 - Discard the supernatant and resuspend with 1 mL ice cold PBS
 - Spin down the cells for 10 min at 13,400 rpm
 - Discard the supernatant and put the pellets on ice until FACS
 - Right before FACS: resuspend with 200 μ L ice cold PBS
- Prepare following tubes:

Tube	[Ab]	PBS-0.1%BSA	Cells (10^7)	Antibody volume to add (μ L)			Ab/tag ratio
				4.52 μ M	1 μ M	200 nM	
1		150 μ L	50 μ L				
2	1 μ M	50 μ L	50 μ L	28.41			1546.6
3	316 nM	150 μ L	50 μ L	15.03			818.2
4	100 nM	150 μ L	50 μ L	4.53			246.6

5	31.6 nM	500 μ L	50 μ L	17.95	216.3
6	10 nM	500 μ L	50 μ L	5.56	67.0
7	3.16 nM	1 mL	50 μ L	16.87	40.6
8	1 nM	1 mL	50 μ L	5.28	12.7
9	316 pM	10 mL	50 μ L	15.92	38.3
10	100 pM	10 mL	50 μ L	5.03	12.1
11	31.6 pM	40 mL	50 μ L	6.33	15.2
12	10 pM	40 mL	50 μ L	2.00	4.8

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- Prepare FACS samples:
 - Spin down the cells for 10 min at 13,400 rpm
 - Discard the supernatant and resuspend with 1 mL ice cold PBS-0.1%BSA
 - Spin down the cells for 10 min at 13,400 rpm
 - Discard the supernatant and put the pellets on ice until FACS
 - Right before FACS: resuspend with 200 μ L ice cold PBS-0.1%BSA